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**REMARKS**

In the claim listing above, claims 91 and 99 have been amended. No claims have been added or canceled by this paper. Accordingly, as amended above, claims 91-103 are presented for further examination in this application.

**Claim Amendments**

As just indicated, independent claims 91 and 99 have been identically amended in the claim listing above. In both claims, the recitation "under isostatic conditions of temperature, buffer and ionic strength" has been deleted from step (c) and moved to the preamble. In addition, a wherein clause has been inserted at the end of each of claims 91 and 99. The inserted wherein clause recites *wherein said steps (a) through (d) are carried out under isostatic conditions of temperature, buffer and ionic strength.*

Entry of the above amendments and new claim listing is respectfully requested.

**Withdrawn Rejection and Accepted Terminal Disclaimer**

Applicants acknowledge with appreciation the indication in the Office Communication (page 2) that the previous rejections under 35 U.S.C. §103(a) have been withdrawn, and that the submitted Terminal Disclaimer has been reviewed, accepted and recorded.

**The Rejection Under 35 U.S.C. §102**

Claims 91-94 and 97-98 stand rejected under 35 U.S.C. §102(b) as being anticipated by Scheele (US Patent 5162209, November 10, 1992). According to the Office Communication (pages 3-5):

With regard to Claim 91, step a, Scheele teaches providing a first

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DNA strand from genomic DNA (e.g. a nucleic sample containing the sequence of a specific sample) (column 3 lines 25 and Column 5 lines 30-35).

With regard to step b, Scheele teaches contacting the DNA with dNTPs (e.g. un modified nucleic acid precursors) (Column 4 lines 20-22). Scheele et al. teaches a primer sequence which is substantially complementary to the distinct sequence of said specific nucleic acid (Column 3 lines 33-40). Scheele teaches a method of adding E. coli DNA polymerase I. (e.g. effective amount of nucleic acid producing catalyst) (column 4 lines 14-15).

Scheele, teaches a primer comprising an RNA segment (Column 3 lines 33-40). Scheele teaches a method of adding E. coli DNA polymerase I (e.g. effective amount of nucleic acid producing catalyst) (column 4 lines 14-15). Scheele teaches a method of adding RNase H (Column 4 lines 25).

With regard to step c, Scheele teaches a method of carrying out synthesis in the presence of the RNA primer to generate a polynucleotide comprising an RNA/DNA hybrid (Column 3 lines 30-40 and Figure 5). The instant specification does not define isostatic conditions of temperature, buffer and ionic strength. Scheele teaches combining the primer and the DNA molecule in a reagent solution at a particular heat with buffers that have a particular ionic strength to produce at least one copy of the DNA molecule by extension of the primer (Column 8 lines 1-15). Therefore Scheele teaches a method of allowing the mixture to react under isostatic condition of temperature, buffer, and ionic strength.

With regard to step d, Scheele teaches a method of digesting the substrate with RNase H to remove the ribonucleic acid segment of the extended primer (Column 3 lines 30-40, column 4 lines 25-28, and Figure 5). Scheele teaches that the method can be adapted to permit amplification of the sample of dsDNA by PCR methodology (column 8 lines 58-60). Scheele teaches that the reagents of the RNA primer, DNA target, dNTP5 and Taq are placed into a PCR machine with the appropriate number of PCR temperature cycles (column 8 lines 65-69). Therefore in a PCR cycle the resultant dsDNA of step c would be denatured such that multiple copies of the DNA could be copied from the DNA of interest.

With regard to Claims 92-93, Scheele et al. teaches a method of removing the primer portion with RNase H (e.g. digestion with an enzyme) (column 4 lines 24-26).

With regard to Claim 94, Scheele et al. teaches a primer comprising an RNA segment (Column 3 lines 33-40).

With regard to Claim 97, Scheele et al teaches that the primer is

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comprises of a tail which is composed of nucleoside triphosphates (column 3 Table I and Column 4 lines 5-10). Scheele et al. teaches that that the triphosphate was radiolabelled and as such was modified (column 8 lines 35-40).

With regard to Claim 98, Scheele teaches that the primer includes a portion of nucleotides that is complementary to an oligonucleotide tail added to the 3' end of the target DNA template (column 3 lines 25-35). Therefore the primers include a sequence which is complementary to the tail and therefore not complementary to the sequence of the DNA molecule of interest (e.g. the tail is added to the DNA target of interest but the tail is not considered the DNA which is of interest to be amplified) (Figure 5 and column 3 lines 25-40). Therefore Scheele et al. teaches primers which comprises about 1 to about 200 noncomplementary nucleotides.

The anticipation rejection is respectfully traversed.

In response, Applicants wish to point out that no mention in the Office Communication that the present claims have a requirement that the primers be chemically modified, a condition that is not described in the cited Scheele patent. Although the instant rejection (page 4) contains a remark that Scheele used radioactively labeled primers, citing column 8, lines 35-40, Applicants believe this to be a misreading because first, the presence of radioactively labeled nucleotides in a primer would not lead persons skilled in the art to consider the primers to be "modified" primers. Other than being a different isotope, there is a phosphorus moiety at each site, whether it is a normal or radioactive version of P, thereby giving the labeled primer the same chemical properties as an unlabeled primer. Second, a close reading of the cited section in Scheele reveals that it is with regard to a riboG tail hybridized to a poly(dC) tail as depicted in Figure 5. Thus, it can be seen that Scheele's primer is not labeled (or modified), but rather, that the element is a radioactively labeled template used for binding and extension. After the extension reaction, digestion of the unlabeled primer by RNase H renders the Poly(dC) in single-stranded form that is a substrate for exonucleolytic digestion by T4. As such, Scheele's conclusion on lines 39-42, column

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8, was a measurement of the removal of radioactively labeled template rather than the primer.

Furthermore, Applicants point out that the present invention and claims have been amended to reflect the limitation that all of the reaction steps are taking place under isostatic conditions. Thus, binding, extension, removal and binding of a new primer on a regenerated primer binding site are taking place simultaneously in the method of claim 91 (as well as claim 99 which is not anticipated according to the Office Communication). Furthermore, although Scheele uses an RNase H step to remove primer sequences, the nucleic acid sequences that are thereby rendered single-stranded are not used for new primer binding events as required by the present claims. Indeed, Scheele teaches away from this aspect of the present invention and claims because he also teaches the simultaneous presence of a nuclease that will digest away the sequences that could otherwise provide a primer binding site. The RNase H digestion step disclosed by Scheele also does not provide means for amplification, but is only used to render the double-stranded nucleic acid into a blunt-ended form. The Office Communication cites a variation that was also described by Scheele where PCR is used to provide amplification, followed by an RNase H step after the completion of the amplification steps. Applicants respectfully point out, however, that the thermal denaturation step that is part of the PCR reaction does not remove a portion of the primer, but instead removes the entire extended nucleic acid. Applicants have nevertheless clarified the nature of the method of the present claims to specifically point out that all of the steps of the reaction take place under isostatic conditions, where the term isostatic condition is understood to mean that conditions of temperature, buffer and ionic strength remain the same during the course of the method of claim 91 (as well as claim 99 which has not been rejected for anticipation). This limitation clearly distinguishes the present invention away from any considerations of similarity to PCR which uses a series of fluctuations in temperatures to enable binding/extension steps separately from denaturation steps.

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With regard to page 3 of the Office Communication, it is pointed out that steps (a), b) and (c) take place under isostatic conditions in Scheele. As noted above, however, the present claims have been amended to specifically claim that all 4 steps [(a), (b), (c) and (d)] are taking place together under isostatic conditions. This is a limitation that altogether lacking in Scheele, because first, Scheele either uses RNase H after an extension and there is no subsequent primer binding as required by the claim or second, Scheele uses PCR followed by an RNase H step which is also outside of the claim as amended.

In view of the above claim amendments and foregoing remarks, Applicants respectfully request reconsideration and withdrawal of the anticipation rejection.

#### Commonality of Ownership

Applicants assert that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made.

#### The First Rejection Under 35 U.S.C. §103

Claims 95-96 stand rejected under 35 U.S.C. §103(a) as being unpatentable over Scheele (US Patent 5162209 November 10, 1992) in view of Gelfand et al. (US Patent 5374553 December 20, 1994). According to the Office Communication (pages 6-8):

Scheele teaches providing a first DNA strand from genomic DNA (e.g. a nucleic sample containing the sequence of a specific sample) (column 3 lines 25 and Column 5 lines 30-35).

Scheele teaches contacting the DNA with dNTP5 (e.g. unmodified nucleic acid precursors) (Column 4 lines 20-22). Scheele et al. teaches a primer sequence which is substantially complementary to the distinct sequence of said specific nucleic acid (Column 3 lines 33-40). Scheele teaches a method of adding E. coli DNA polymerase I (e.g. effective amount of nucleic acid producing catalyst) (column 4 lines 14-15).

Scheele. teaches a primer comprising an RNA segment (Column 3 lines 33-40). Scheele teaches a method of adding E. coli DNA polymerase I (e.g. effective amount of nucleic acid producing catalyst) (column 4 lines

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14-15). Scheele teaches a method of adding RNase H (Column 4 lines 25).

Scheele teaches a method of carrying out synthesis in the presence of the RNA primer to generate a polynucleotide comprising an RNA/DNA hybrid (Column 3 lines 30-40 and Figure 5). The instant specification does not define isostatic conditions of temperature, buffer and ionic strength. Scheele teaches combining the primer and the DNA molecule in a reagent solution at a particular heat with buffers that have a particular ionic strength to produce at least one copy of the DNA molecule by extension of the primer (Column 8 lines 1-15). Therefore Scheele teaches a method of allowing the mixture to react under isostatic condition of temperature, buffer, and ionic strength.

Scheele teaches a method of digesting the substrate with RNase H to remove the ribonucleic acid segment of the extended primer (Column 3 lines 30-40, column 4 lines 25-28, and Figure 5). Scheele teaches that the method can be adapted to permit amplification of the sample of dsDNA by PCR methodology (column 8 lines 58-60). Scheele teaches that the reagents of the RNA primer, DNA target, dNTP5 and Taq are placed into a PCR machine with the appropriate number of PCR temperature cycles (column 8 lines 65-69). Therefore in a PCR cycle the resultant dsDNA of step c would be denatured such that multiple copies of the DNA could be copied from the DNA of interest.

However, Scheele et al. does not teach the modification of the primers to comprise an isosteric configuration of heteroatoms.

With regard to Claims 95-96, Gelfand et al teaches that to avoid primer degradation in PCR, phosphorothioate (e.g. comprises of sulfur heteroatom) can be added to the 3' ends of the primers to allow the primers to be more resistance to degradation (Column 13 lines 15-20).

Therefore it would be *prima facie* obvious to modify the *in vitro* translation method of Scheele et al. to have a phosphorothioate (sulfur heteroatoms) on the 3' end of the primers as taught by Gelfand et al. in order to maintain the primers during the PCR step of Scheele et al (column 8 lines 58-60) to produce multiple copies of the nucleic acid of interest. The ordinary artisan would be motivated to modify the primer of Scheele et al. to include the phosphorothioate (sulfur heteroatoms) of Gelfand et al., because Gelfand et al. teaches that the addition of phosphorothioate to the primers ends allows the primers to be more resistant to degradation (column 13 lines 15-20). Therefore the ordinary artisan would be motivated to modify the primers of Scheele et al. to include the phosphorothioate (sulfur heteroatom) of Gelfand et al. because primers resistant to degradation can be maintained longer in a PCR and therefore more copies of the original nucleic acid may be

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produced.

The first obviousness rejection is respectfully traversed.

In response, Applicants respectfully point out that the addition of Gelfand to Scheele is insufficient to reach the present invention and claims. Because Scheele does not disclose or suggest the present invention for reasons advanced in the anticipation rejection above, then as a matter of logic, the addition of Gelfand does not render dependent claims 95 and 96 obvious. Quite simply, Gelfand does not cure the deficiencies of Scheele to reach either or both dependent claims. Indeed, Gelfand repeats some of the same deficient practices of Schele in that the former is also concerned with PCR thermal cycling rather than an isostatic mode of amplification, as set forth in the present invention and claims.

In view of the above claim amendments and foregoing remarks, Applicants respectfully request reconsideration and withdrawal of the first obviousness rejection.

#### The Second Rejection Under 35 U.S.C. §103

Claims 99-103 stand rejected under 35 U.S.C. §103(a) as being unpatentable over Scheele (US Patent 5162209 November 10, 1992) in view of Reischl et al. (US Patent 5474916 December 12, 1995). According to the Office Communication (pages 8-11):

With regard to Claim 99, step a, Scheele teaches providing a first DNA strand from genomic DNA (e.g. a nucleic sample containing the sequence of a specific sample) (column 3 lines 25 and Column 5 lines 30-35).

With regard to step b, Scheele teaches contacting the DNA with dNTPs (e.g. unmodified nucleic acid precursors) (Column 4 lines 20-22). Scheele et al. teaches a primer sequence which includes a portion of nucleotides that is complementary to an oligonucleotide tail added to the 3' end of the target DNA template (column 3 lines 25-35). Therefore the primers include a sequence which is complementary to the tail and therefore not complementary to the sequence of the DNA molecule of interest (e.g. the tail is added to the DNA target of interest but the tail is

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not considered the DNA which is of interest to be amplified) (Figure 5 and column 3 lines 25-40). As such the primer comprises at least one noncomplementary nucleotide. However Scheele et al. does not teach the formation of a loop structure.

Scheele teaches a method of adding E. coli DNA polymerase I (e.g. effective amount of nucleic acid producing catalyst) (column 4 lines 14-15).

Scheele teaches a primer comprising an RNA segment (Column 3 lines 33-40). Scheele teaches a method of adding E. coli DNA polymerase I (e.g. effective amount of nucleic acid producing catalyst) (column 4 lines 14-15). Scheele teaches a method of adding RNase H (Column 4 lines 25).

With regard to step c, Scheele teaches a method of carrying out synthesis in the presence of the RNA primer to generate a polynucleotide comprising an RNA/DNA hybrid (Column 3 lines 30-40 and Figure 5). The instant specification does not define isostatic conditions of temperature, buffer and ionic strength. Scheele teaches combining the primer and the DNA molecule in a reagent solution at a particular heat with buffers that have a particular ionic strength to produce at least one copy of the DNA molecule by extension of the primer (Column 8 lines 1-15). Therefore Scheele teaches a method of allowing the mixture to react under isostatic condition of temperature, buffer, and ionic strength.

With regard to step d, Scheele teaches a method of digesting the substrate with RNase H to remove the ribonucleic acid segment of the extended primer which would include the tail portion of the primer (Column 3 lines 30-40, column 4 lines 25-28, and Figure 5). Scheele teaches that the method can be adapted to permit amplification of the sample of dsDNA by PCR methodology (column 8 lines 58-60). Scheele teaches that the reagents of the RNA primer, DNA target, dNTPs and Taq are placed into a PCR machine with the appropriate number of PCR temperature cycles (column 8 lines 65-69). Therefore in a PCR cycle the resultant dsDNA of step c would be denatured such that multiple copies of the DNA could be copied from the DNA of interest.

With regard to Claims 100-101, Scheele et al. teaches a method of removing the primer portion with RNase H (e.g. digestion with an enzyme) (column 4 lines 24-26).

With regard to Claim 102, Scheele et al. teaches a primer comprising an RNA segment (Column 3 lines 33-40).

With regard to Claim 103, Scheele teaches that the primer includes a portion of nucleotides that is complementary to an oligonucleotide tail added to the 3' end of the target DNA template (column 3 lines 25-35). Therefore the primers include a sequence which is complementary to the

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tail and therefore not complementary to the sequence of the DNA molecule of interest (e.g. the tail is added to the DNA target of interest but the tail is not considered the DNA which is of interest to be amplified) (Figure 5 and column 3 lines 25-40). Therefore Scheele et al. teaches primers which comprises about 1 to about 200 noncomplementary nucleotides.

However, Scheele et al. does not teach a method wherein the primers form a loop structure which is removed from the complementary copy.

With regard to Claim 99, Reischl et al teaches a method wherein the primer comprises a loop structure at the end which is used by the polymerase as an initiator site to synthesize the DNA structure (Column 12 lines 60-66-Column 13 lines 1-10). Therefore it would be obvious to modify the tail portion of the primer of Scheele et al. to include a loop structure which upon hybridization to the template would be used as an initiator site for synthesis. Scheele et al. teaches that the primer is then digested with RNASH and therefore the loop structure would be removed from the complementary strand.

Therefore it would be prima facie obvious to one of ordinary skill in the art to modify the teachings of Scheele et al. such that a primer with a loop on the end is used to synthesize DNA as taught by Reischl et al. The ordinary artisan would be motivated to add a loop to the primer structure of Scheele et al. because Reischl et al. teaches that this loop contains a region for the polymerase to start making a complementary copy of the structure (Column 12 lines 60-66-Column 13 lines 1-10). Therefore the ordinary artisan would be motivated to have a loop structure so that the polymerase has an initial starting point for transcription and removing the loop structure along with the rest of the primer with RNase H after synthesis in order to allow for a new strand to be synthesized.

The second obviousness rejection is respectfully traversed.

In response, Applicants respectfully submit that even the addition of Reischl et al. to Scheele's disclosure would not have rendered the present invention and claims obvious. In the first place, although Scheele teaches that digestion by RNase H of an RNA primer can render a template single-stranded, he does not teach the use of the single-stranded portion created by this process as being used as a primer binding site. In claim 99, the presence of the non-complementary sequence creates a loop, i.e., there are sequences flanking each side of the mis-matched portion. In contrast to the

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present invention and claims, (and as also noted in the instant rejection), the presence of the "tail" disclosed in the Scheele patent would not be considered to be a loop since only one side of the single-stranded region is hybridized to a complementary segment. In addition, as described above, removal of any and all sequences by Scheele does not generate a primer binding site that is re-used by another primer (with the exception of application non-isostatic conditions such as PCR).

On page 10, the Office Action contends that this deficiency is remedied by inclusion of the methods of Reischl. Applicants would respectfully point out, however, that it is altogether uncertain how RNase H would remove the loop portion because RNaseH has no affinity for single-stranded DNA and only uses double-stranded nucleic acids (RNA/DNA hybrids) as substrates. Applicants would also like to point out that Resichel has a pre-existing loop in its nucleic acid primer. In contrast to the secondary reference, the present invention and claims recite "upon hyridization to said specific nucleic acid at least one loop structure is formed . . ." It can be clearly seen, therefore, that loop formation in the present invention and claims is a consequence of hybridization. Furthermore, because the combination of Scheele and Reischl et al. is proposed to result in a primer with a segment that is complementary to the analyte, and an additional non-complementary tail that has a loop structure, there is no suggestion or explanation in either cited document why the digestion of a tail that contains a non-complementary loop would promulgate another primer binding event.

In view of the foregoing remarks, Applicants respectfully request reconsideration and withdrawal of the second obviousness rejection.

**Submission Of Information Disclosure Statement**

Applicants are concurrently submitting their IDS as evidenced by the two sheets of listed documents on Form PTO/SB/08A (01-08) [1 sheet] and Form PTO/SB/08B (01-08) [1 sheet], including three non-U.S. patent documents.

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It is respectfully requested that the documents listed in their concurrently filed IDS be made of record in this application and that the Examiner consider each document for its possible importance in determining whether to allow any claims in this application.

Early and favorable action is respectfully requested.

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**SUMMARY AND CONCLUSIONS**

In the above claim listing for this application, only claims 91 and 99 have been amended. No claims have been added or canceled by this paper.

No claim fee is believed due for this paper because the same number of claims are presented as previously paid for claims. This paper is also accompanied by a Request For Extension Of Time (3 months), Applicants' Information Disclosure Statement, and authorization for the fees therefor. No other fee or fees are believed due in connection with this paper or the accompanying extension request. In the event that any other fee(s) is/are due in connection with this filing, however, the Patent and Trademark Office is hereby authorized to charge the amount of any such fee(s) to Deposit Account No. 05-1135, or to credit any overpayment thereto.

If a telephone call would be helpful in the processing of this paper or this application, Applicants' undersigned attorney requests that he be contacted at the numbers below.

Respectfully submitted,



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